Epidemiology of Clostridium difficile infections in the Netherlands and Europe: implications for surveillance and control

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Chapter 4

Clinical and microbiological characteristics of *Clostridium difficile* infection among hospitalized children in the Netherlands

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Background. Little is known about paediatric *Clostridium difficile* infection (CDI) epidemiology. We describe the clinical and microbiological characteristics of CDI among hospitalized children in the Netherlands.

Methods. Between May 2009 and May 2015, 26 hospitals registered characteristics of paediatric (aged 2–18 years) and adult (aged ≥18 years) CDI in a national sentinel surveillance study. Routine polymerase chain reaction (PCR) ribotyping and multiple-locus variable-number tandem-repeat analysis (MLVA) of selected strains was performed. Paediatric and adult results were compared using proportion and 95% confidence interval (CI). Time trend of paediatric CDI was evaluated using a mixed-effect Poisson model.

Results. Paediatric CDIs were reported in 17 of the 26 participating hospitals (n = 135; 3% of all CDIs); the monthly number was constant over time. The median age of paediatric cases was 10 years (interquartile range, 4.7–14.5 years). Fifty-five percent of the children had community onset and 31% had severe CDI. Compared with adults (n = 4,556), complication and mortality rates were lower. *Clostridium difficile* PCR ribotype 265 (toxin A negative, B positive) was most prevalent in children (15%; 95% CI, 8.8%–24.0%) but rarely found in adults (1%; 95% CI, 0.9%–1.6%). This strain was rarely found in other countries, except for Belgium. MLVA showed genetic relatedness between three-fourths of paediatric and adult ribotype 265 strains, without a clear epidemiological link.

Conclusions. Paediatric CDI in hospitals has remained stable over the last 6 years and resulted in fewer complications than for adult CDI. Further studies are needed to elucidate the source and epidemiology of PCR ribotype 265, primarily found in children.

Introduction

Clostridium difficile is a commensal bacterium in newborns (30%–35% positive) and infants aged up to 2 years (10%–15% positive) [1 – 3]. Infants are considered insensitive to free *C. difficile* toxins in the intestinal tract for several reasons, including lack of mature toxin-binding receptors on epithelial cells and incomplete cellular uptake of the toxins and/or protecting microbiome composition [1, 4]; however, clear scientific evidence is lacking.

Clostridium difficile is potentially harmful for children aged >2 years [5], though the presence of toxin-producing *C. difficile* in stools is not conclusive for diagnoses [6 – 8]. Children with a presumed *C. difficile* infection (CDI) have a 1.2 – 2 times higher in-hospital mortality risk and prolonged hospitalizations compared with matched controls [9, 10]. However, the absolute mortality risk, primarily ranging from 0% to 1.5% [10 – 16] and from 3% to 5% in certain studies [9, 17, 18], is much lower than for adults [11].

In the last decade, the reported incidence of paediatric CDI in both community and hospitals settings in the United States increased [10, 14, 17, 19]. Most children who develop CDI have underlying comorbidities [9, 13, 17]. Recent use of anti-biotic drugs, the presence of gastrointestinal feeding devices, prior hospitalization, and comorbidities, such as malignancies, inflammatory bowel disease, and organ transplantation, are the main risk factors [10, 20, 21].

Here, we describe the clinical and microbiological characteristics of CDI in hospitalized children in the Netherlands as part of a CDI national sentinel surveillance study and compare these results to those of adult cases. Our aim is to determine the burden of paediatric CDI and to determine if additional strategies to prevent, diagnose, and treat CDI in children are needed.

Methods

STUDY DESIGN

We included all CDIs reported between May 2009 and May 2015 by 26 Dutch hospitals that participate in a national sentinel surveillance study (no ethical approval needed). Hospitals were requested to register all hospitalized patients who fulfilled the clinical CDI definition listed below. Children aged <2 years were excluded. For each CDI patient, information on the history of CDI <8 weeks prior to the current infection, the location of onset (community or hospital), and CDI severity was collected. Antibiotic use prior to CDI onset (for treatment of infections other than CDI) at time of or during admission was registered. Polymerase chain reaction (PCR) ribotyping was used to characterize *C. difficile* isolates. Thirty days after diagnosis, complications, including surgery and admission to an intensive care unit due to CDI and mortality, were assessed. Additionally, hospitals reported the primary CDI diagnostic test applied during participation (possibly limiting surveillance sensitivity).

DEFINITIONS

A paediatric CDI was defined as the occurrence of diarrhoea (3 or more loose stools per day for 2 subsequent days) or a toxic megacolon and a positive test for a toxin-producing *C. difficile* or presence of pseudomembranous colitis in children aged 2–18 years. Other causes of diarrhoea were excluded (by chart review and/or results of diagnostic tests for other enteropathogens). Patients aged ≥18 years who fulfilled the same criteria were categorized as "adults" with CDI. A case was considered as healthcare-onset CDI (HO-CDI) if symptoms started in a hospital or long-term care facility and as community-onset CDI (CO-CDI) if symptoms started at home. Severe CDI (for both children and adults) was defined as either the presence of bloody diarrhoea and/or pseudomembranous colitis, and/or diarrhoea accompanied by dehydration (as judged by the treating physician) and/or hypoalbuminemia (<20 mg), and/or fever (≥38.0°C) with leucocytosis (>15.0 × 10^{9}/L). Mortality was considered as "contributable to CDI" if other comorbidities would not have caused death or "partly contributable to CDI" if both CDI and other comorbidities caused death.

LABORATORY METHODS

The primary (first) diagnostic test of each participating hospital was variable for many hospitals (Supplementary Table 1). Positive samples were sent to the central laboratory (Leiden University Medical Centre, the Netherlands) for typing. Real-time PCR was used to identify the GDH (glutamate dehydrogenase) gene specific to *C. difficile* [22], and PCR ribotyping was used to characterize the isolates [23].

To study relatedness of the predominant paediatric PCR ribotype 265 in more detail, multiple-locus variable-number tandem-repeat analysis (MLVA) was applied [24]. We selected all ribotype 265 strains from children with CDI included in this

study and from 2 preceding and 2 successive adult ribotype 265 cases in the same hospital (also from cases typed apart from the sentinel surveillance study). MLVA results were visualized in a minimum spanning tree using BioNumerics software, version 7.1 (Applied Maths, Saint-Martens-Latem, Belgium). All ribotype 265 isolates were tested for deletions in the *TcdA* gene using PCR [25]. The genetic relatedness of PCR ribotype 265/ sequence type 88 to other *C. difficile* types was visualized in a phylogenetic tree, using multilocus sequence typing (MLST) results of the *Clostridium difficile* MLST Databases. Further, we requested international collaborators (eg, the ESCMID Study Group for *Clostridium difficile*, founders of the *Clostridium difficile* MLST Databases, Webribo, and the US Centers for Disease Prevention and Control) to verify the presence of ribotype 265 in their databases by sharing the capillary electrophoresis PCR ribotyping peak file.

STATISTICAL ANALYSES

Categorical or binary variables were reported as frequencies and percentages and compared based on their 95% confidence intervals (CIs). Continuous variables were reported as medians and the interquartile ranges (IQRs). Quantile regression was used to assess associations between age and covariables. To evaluate a time trend of paediatric CDI, the monthly number of reports was analysed using a mixed-effect Poisson model, allowing random effects per hospital, to account for clustered data. Additionally, we corrected for the type of primary diagnostic test applied (categorized in free toxin detection, tests including GDH detection, or Nucleic acid amplification test (NAAT)). Complete case analysis was performed, except for 1 sensitivity analysis on CO-CDI vs HO-CDI and prior antibiotic usage. Data were analysed using Stata software, version 12.1 (StataCorp LP, College Station, Texas).

Results

PAEDIATRIC CDI REPORTING AND TIME TRENDS

Between May 2009 and May 2015, 4691 CDIs were reported. A total of 135 paediatric CDIs (3% of all CDIs) were reported by 17 hospitals (65%). There were large interhospital differences in the proportion of paediatric CDIs, especially when university hospitals (range, 1.8%–14.3%) were compared with nonuniversity hospitals (range, 0%–7.7%; Supplementary Table 1). No CDI outbreaks on paediatric wards were reported. The number of paediatric CDIs per month was stable according to mixed-effect Poisson modelling ($P = .578$), also when correcting for the type of primary diagnostic test applied ($P = .145$).

CLINICAL CHARACTERISTICS AND 30-DAY OUTCOME

The median age of children with CDI was 10 years (IQR, 4.7–14.5 years) and similar for children with CO-CDI and HO-CDI (11 vs 10 years [$P = .84$]). Fifty-three percent of the children with CO-CDI received non-CDI antibiotics prior to CDI onset (28/53 [95% CI, 39.1%–66.6%]) compared with 81% of the children with HO-CDI (43/53 [95% CI, 70.4– 91.9%]), though information on antibiotic use was missing for 29 cases. If we assumed that all children with missing information either used or did not use non-CDI antibiotics prior to CDI onset, the difference persisted (66% vs 84% and 38% vs 70%, respectively).

A total of 39 children (31%, data for 8 children missing) met the criteria for having severe CDI. Eighteen of these children were dehydrated or had hypoalbuminemia (46%), 16 had bloody diarrhea (41%), 10 had fever and leucocytosis (26%), and 5 had pseudomembranous colitis (13%). Females had severe CDI more often than males (45% [95% CI, 31.8%–58.2%] vs 19% [95% CI, 9.5%–28.2%]).

A complicated course within 30 days after diagnosis was reported for at least 3 children (3%, data of 37 children missing). One 6-year-old female was admitted to the intensive care unit suffering from CDI with dehydration/hypoalbuminemia and fever with leucocytosis. Two males died within 30 days after diagnosis due to causes other than CDI.

PAEDIATRIC CDI COMPARED WITH ADULT CDI

In Table 1, clinical data of children are compared with those of adult CDI patients (n = 4556). CO-CDI was more com-mon in children than in adults (55% [95% CI, 46.4%–63.2%] vs 33% [95% CI, 31.8%–34.6%]). Severe CDI was more frequently observed in children than in adults (31% [95% CI, 22.7%–38.8%] vs 23% [95% CI, 22.1%–24.6%]. In contrast to adults, CDI-related mortality did not occur in children (0% vs 4% [95% CI, 3.0%–4.2%].

Table 1. Clinical Characteristics and 30-Day Outcome of Children With *Clostridium difficile* Infection Compared With Adults

Patient demographics and course of CDI	Paediatric cases, n = 135 (%)	Adult cases, n = 4556 (%)
Age category, y		
2–5	41 (30.4)	-
6–9	26 (19.3)	-
10–13	30 (22.2)	-
14–17	38 (28.1)	-
Male gender	74 (54.8)	2209 (48.5)
Previous CDI		
Yes, clinical presentation only	1[a] (1.2)	173 (6.5)
Yes, clinical presentation and positive test	19[a] (23.2)	474 (17.8)
No, no clinical presentation	42[a] (51.2)	1408 (52.9)
No, negative test	20[a] (24.4)	608 (22.8)
Days to diagnosis of hospital-onset CDI[b]	2 (1–8)	2 (1–6)
Community onset of symptoms	74 (54.8)	1480 (33.2)
Prior antibiotic use[c]	71[d] (67.0)	2674 (70.2)
Severe CDI	39[e] (30.7)	968 (23.3)
30-day outcome		
Complicated course	3[f] (3.1)	568 (15.4)
Overall mortality	2[f] (2.0)	507 (13.7)
CDI-related mortality	0[f] (0)	133 3.6)

Abbreviation: CDI, *Clostridium difficile* infection.

[a] Data missing for 53 children.
[b] Expressed as median number of days and the interquartile range.
[c] At time of diagnosis or during admission for treatment of infections other than CDI.
[d] Data missing for 29 children.
[e] Data missing for 8 children.
[f] Data missing for 37 children.

PCR RIBOTYPING DISTRIBUTION

For 113 of 135 paediatric CDIs (84%), a stool sample or *C. difficile* isolate was sent to the reference laboratory for PCR ribotyping. *Clostridium difficile* was detected in 98 samples (n = 5 culture negative; n = 10 *Clostridium* species but not *C. difficile*). In total, 36 different *C. difficile* PCR ribotypes were identified. Table 2 illustrates the ribotyping distribution of paediatric CO-CDI and HO-CDI compared with adults (information on CDI onset and typing results for 3573/4556 adults available). Ribotype 265 was most prevalent in children (15% [95% CI, 8.8%–24.0%]) but rarely found in adults (1% [95% CI, 0.9%–1.6%]). Ribotype 014/020 was commonly found in both children and adults (12% [95% CI, 6.5%–20.4%] and 15% [95% CI, 13.9%–16.3%], respectively). Ribotypes 001 and 078/126 were less frequently isolated from children than from adults (5% [95% CI, 1.7%–11.5%] vs 15% [95% CI, 13.7%–16.1%]

and 5% [95% CI, 1.7%–11.5 %] vs 13% [95% CI, 12.3%–14.5%], respectively). Ribotype 027 strain was not found in the paediatric population but was present in 3% of the adult population (n=94 [95% CI, 2.1%–3.2%]). The differences in ribotype distribution were larger when HO-CDI was compared with CO-CDI (Table 2).

PCR RIBOTYPE 265

Paediatric ribotype 265 cases occurred in 7 hospitals located in different regions of the Netherlands (Figure 1). Two-thirds of the cases occurred between March 2012 and March 2013 (n=10), dispersed over several months. Ribotype 265–infected children were younger than those infected by other ribotypes (median age of 4 vs 11 years). Children and adults with a ribotype 265 CDI (n=15 and n=45) did not have more severe CDI com-pared with those infected by other ribotypes (29% vs 31% and 28% vs 24%, respectively) and did not have a higher 30-day mortality risk (0% vs 4% and 14% vs 14%, respectively). None of the ribotype 265–infected adults deaths were partly related to CDI.

MLVA showed that three-fourths of the ribotype 265 strains isolated from children and adults were genetically related (defined as ≤10 summed tandem-repeat difference [24]; Figure 1). Three clonal complexes (defined as ≤2 summed tandem-repeat difference on ≤2 loci [24]) were found, of which 2 complexes included adult and paediatric isolates from different hospitals. Three children had a recurrent ribotype 265 infection (Figure 1). Three ribotype 265 strains that were isolated in 1 university hospital (U3) in October 2012–November 2012 were genetically, but not clonally, related.

Ribotype 265 had an intact toxin B production but lacked toxin A due to a *TcdA* 1.8 kb deletion as ribotype 017 [25] and was negative for binary toxin genes. A phylogenetic tree of multilocus sequence types submitted to the *Clostridium difficile* MLST Databases illustrated the genetic relatedness of ribotype 265 (assigned as sequence type 88) and ribotype 017 (assigned as sequence type 37; Supplementary Figure 2). According to the survey in our international network, ribotype 265 appears to be very uncommon or absent in other countries, except for Belgium (Supplementary Table 2). In Belgium, ribotype 265 was primarily found isolated from children aged <2 years.

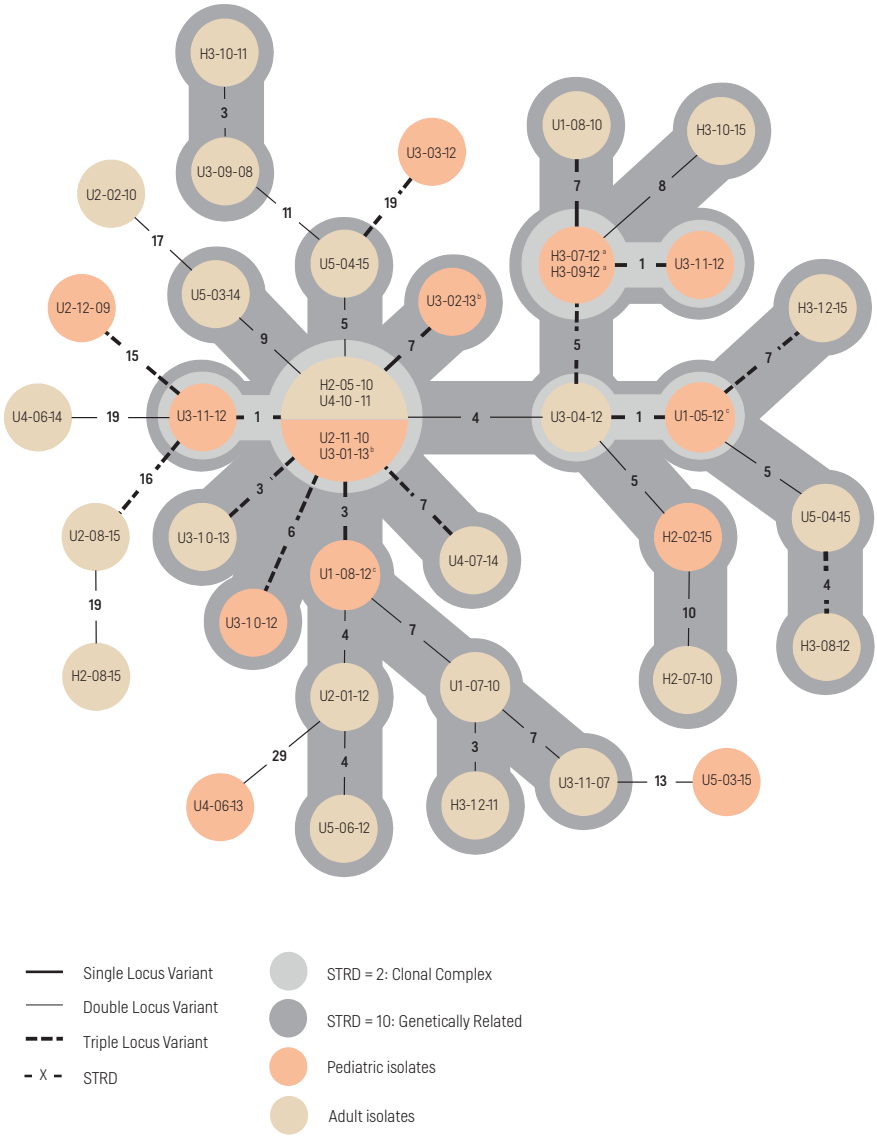
Table 2. Polymerase Chain Reaction Ribotype Distribution of Community Onset and Healthcare Onset *Clostridium difficile* Infection in Hospitalized Children Compared With Hospitalized Adults in the Netherlands

Polymerase chain reaction ribotype	Children				Adults[a]			
	CO, n = 53 (%)		HO, n = 45 (%)		CO, n = 1182 (%)		HO, n = 2391 (%)	
001	2	(3.8)	3	(6.7)	105	(8.9)	427	(17.9)
002	6	(11.3)	2	(4.4)	76	(6.4)	148	(6.2)
005	6	(11.3)	2	(4.4)	54	(4.6)	105	(4.4)
014/020	8	(15.1)	4	(8.9)	179	(15.1)	359	(15.0)
027	-		-		24	(2.0)	70	(2.9)
078/126	4	(7.5)	1	(2.2)	176	(14.9)	302	(12.6)
087	2	(3.8)	2	(4.4)	18	(1.5)	34	(1.4)
106	1	(1.9)	2	(4.4)	6	(0.5)	6	(0.3)
265	4	(7.5)	11	(24.4)	14	(1.1)	31	(1.3)
Other types	20	(37.7)	15	(33.3)	405	(34.3)	748	(31.3)
Unknown	-		3	(6.7)	125	(11.2)	161	(6.7)

The 8 most common ribotypes (n ≥3 isolates) in children were taken as a reference, and ribotype 027 was included for its clinical relevance. A dash indicates a zero.
Abbreviations: CO, community onset; HO, healthcare onset.

[a] Two adults had a mixed infection, the polymerase chain reaction ribotype with the lowest number is reported in the table.

Figure 1 Minimum spanning tree of *Clostridium difficile* polymerase chain reaction ribotype 265 strains isolated from children (in blue; n = 15) and adults (in green; n = 24), according to multiple-locus variable-number tandem-repeat analysis. The STRD is shown between the circles. Dark gray areas represent a cluster of genetically related strains (defined as ≤10 summed tandem-repeat difference), and light gray areas represent clonal complexes (defined as ≤2 summed tandem-repeat difference on ≤2 loci). Each circle specifies the code for the hospital where the patient was located and the month and year of isolation. Superscripts indicate samples that were isolated from identical patients (a, b, and c). Abbreviations: STRD, summed tandem-repeat difference; U, H (U indicates University hospitals and H primary and secondary care hospitals, see Supplementary Table 1).



Discussion

We report the clinical characteristics and PCR ribotypes of paediatric CDI in a large sentinel surveillance study in the Netherlands during a 6-year period.

In contrast with several studies performed in the United States [10, 14, 17, 19] and Italy [26] but in agreement with a recent study in the United Kingdom [24], we did not find an increase in paediatric CDIs over time. The increase in the United States could be related to the high prevalence of NAP1/ribotype 027 or to an increased awareness of the need to test children for CDI, though this is opposed by 1 study [17]. The recent implementation of NAAT in many laboratories may have contributed to this increase [27] by possibly including *C. difficile* carriers as CDIs. It did have a significant effect on paediatric CDI reporting in our analysis and was incorporated into our model. However, both the unadjusted and adjusted models did not indicate an increase in paediatric CDIs.

We found large interhospital differences in the proportion of reported paediatric CDIs, especially between university hospitals and nonuniversity hospitals (5% vs 0.3%). University hospitals indeed treat children with a higher CDI risk, such as those with cancer, as well as organ and bone marrow trans-plantation patients [21]. However, we questioned whether non-university hospitals actually did test for *C. difficile* in children. We contacted 9 hospitals that do not or rarely report paediatric CDI; 1 did not test for CDI in children, but 7 tested on clinical request in children of all ages, and 1 only in patients aged >2 years. There is no guideline for CDI testing in children in the Netherlands (or in Europe) in contrast to the United States [4, 28]. We assume that differing views among both paediatricians and microbiologists on CDI testing in children contributed to interhospital differences.

The clinical characteristics of paediatric CDI found in our study resemble findings of other studies, despite the higher median age of patients in our study [9, 13, 17, 18, 20]. Approximately half of the paediatric cases had CO-CDI, which is in line with 2 studies that used different criteria (29% and 54%) [9, 18], while others found 26% and 71% of paediatric CDI cases to be acquired in the community [13, 15]. Antibiotics are considered to be a risk factor but not a prerequisite for developing paediatric CDI [21]. In our study, 67% of the children received non-CDI antibiotics prior to CDI onset, which is similar to previous studies that showed rates of 61%–74%, depending on the measured time period of exposure [14, 18]. As expected, the percentage exposed to non-CDI antibiotics was lower in paediatric CO-CDI than in HO-CDI.

Compared with adults, we found a high proportion of severe CDI (31%) in children but a lower complication and mortality rate 30 days after diagnosis. Possibly, children are more capable than adults of recovering from severe CDI [21] or possibly

the current severity criteria do not fit the paediatric population [29]. This is supported by a study that revealed that 50% of children with CDI did not require specific CDI treatment, while 76% were classified as "severe CDI" [24]. However, the severity rate in that study was much higher than in our study, and as described in literature [13 – 15].

PCR ribotype 265 (toxin A negative due to a *TcdA* 1.8 kb deletion, toxin B positive, and negative for binary toxin genes) was most prevalent in children and rarely found in adults. Epidemiological and molecular typing data could not elucidate ribotype 265 transmission routes. MLVA showed that three-fourths of the ribotype 265 strains isolated from children and adults were genetically related. There was no clustering according to host (children vs adults), date, or place of isolation. These results suggest ongoing (regional) transmission between the 2 populations, whereas this ribotype may favour younger hosts in particular. In Europe, ribotype 265 was initially observed in Leiden in 2006 and determined to be a new ribotype at the Anaerobe Reference Laboratory in Cardiff (personal communication, Dr Michael Perry) in 2010. In the United States, it was first isolated in 1988 (personal communication, Dr Jane Marsh) using MLST and belongs to the same lineage as ribotypes 017, 047, 088, 130, and 172 (Supplementary Figure 2) [30]. Our international survey showed that this ribotype was rarely found in North America and Europe, except for the neighbouring Belgium where most were isolated from children aged <2 years. Although we did not include children aged <2 years in our study (as many other studies), these findings support our hypothesis that the ribotype favours younger hosts and that transmission may be restricted to certain countries.

After ribotype 265, ribotype 014/020 was most often detected in children (12%), consistent with previous studies where NAP4/ PCR ribotype 014 was predominant in 26% and 24% [13, 31]. Another study showed PCR ribotype 014 to be predominant in infants (aged <2 years) in 25% in Spain [32]. Our study, as well as a German study [31], indicated an absence of ribotype 027 in children in contrast to adults, though both studies typed a limited number of children. In contrast, NAP1/ ribotype 027 was found in 11% and 23% of the paediatric CDI cases in the United States and Canada [13, 15], including community-acquired CDI [15]. In a different single-center US cohort, NAP1/ribotype 027 was found in <1% of the children, opposed to 31% of adults in the same area [33]. These geographical differences may be due to diverse antibiotic exposure (e.g., fluoroquinolones), infection prevention measures, or infection pressure in the community.

Our study has several limitations. CDI testing of children was not as well standardized as for adults and may have resulted in a general underestimation of the burden of paediatric CDI. Our definition of community-onset CDI, which was introduced in 2009 for feasibility reasons, differs from international guide-lines and hampers benchmarking. Our sample size was relatively small, hampering

multivariate analysis, and we had high levels of missing data for some variables. Age-specific denominator data to calculate age-stratified incidence rates were not available, though we evaluated the monthly number of paediatric CDIs using a mixed-effect Poisson model, corrected for the primary diagnostic method applied, and showed that no variation in time was present. In addition, the number of paediatric admissions in the Netherlands [34] did not change over time. The absence of epidemiological links between ribotype 265 cases may be caused by incomplete sampling (eg, nondiagnosed children and asymptomatic carriers) and the lack of detailed patient data, although a dispersion in time and location was evident.

In conclusion, we did not observe an increase in the monthly number of reported paediatric CDIs over a 6-year period. *Clostridium difficile* ribotype 265 was more prevalent in children than in adults, without a clear explanation. Future prospective paediatric studies are needed to obtain more detailed information on CDI risk factors, transmission, and treatment in children and to confirm and elucidate why some PCR ribotypes are more or less abundant compared with adults.

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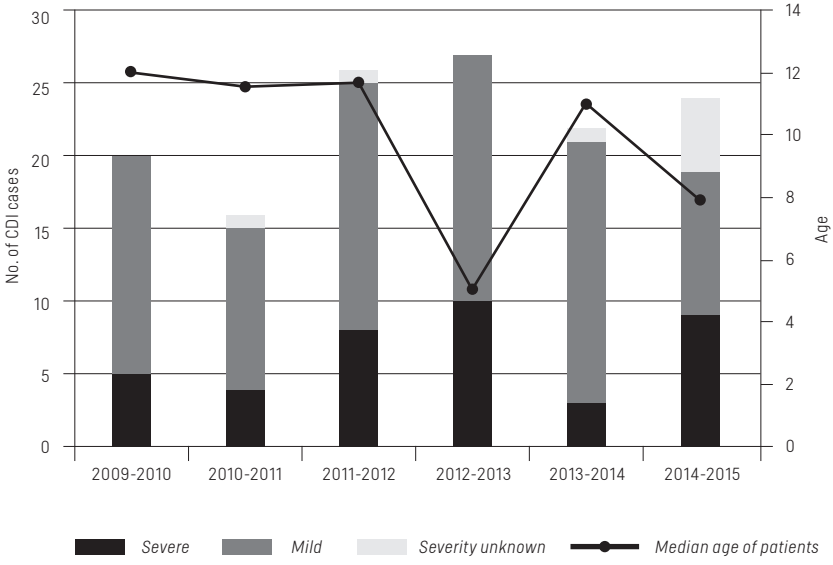
Supplementary figures

Supplementary Figure 1 Number of reported paediatric CDIs per 12-month time period, stratified by CDI severity. The line illustrates the median age of the patients for each time period.

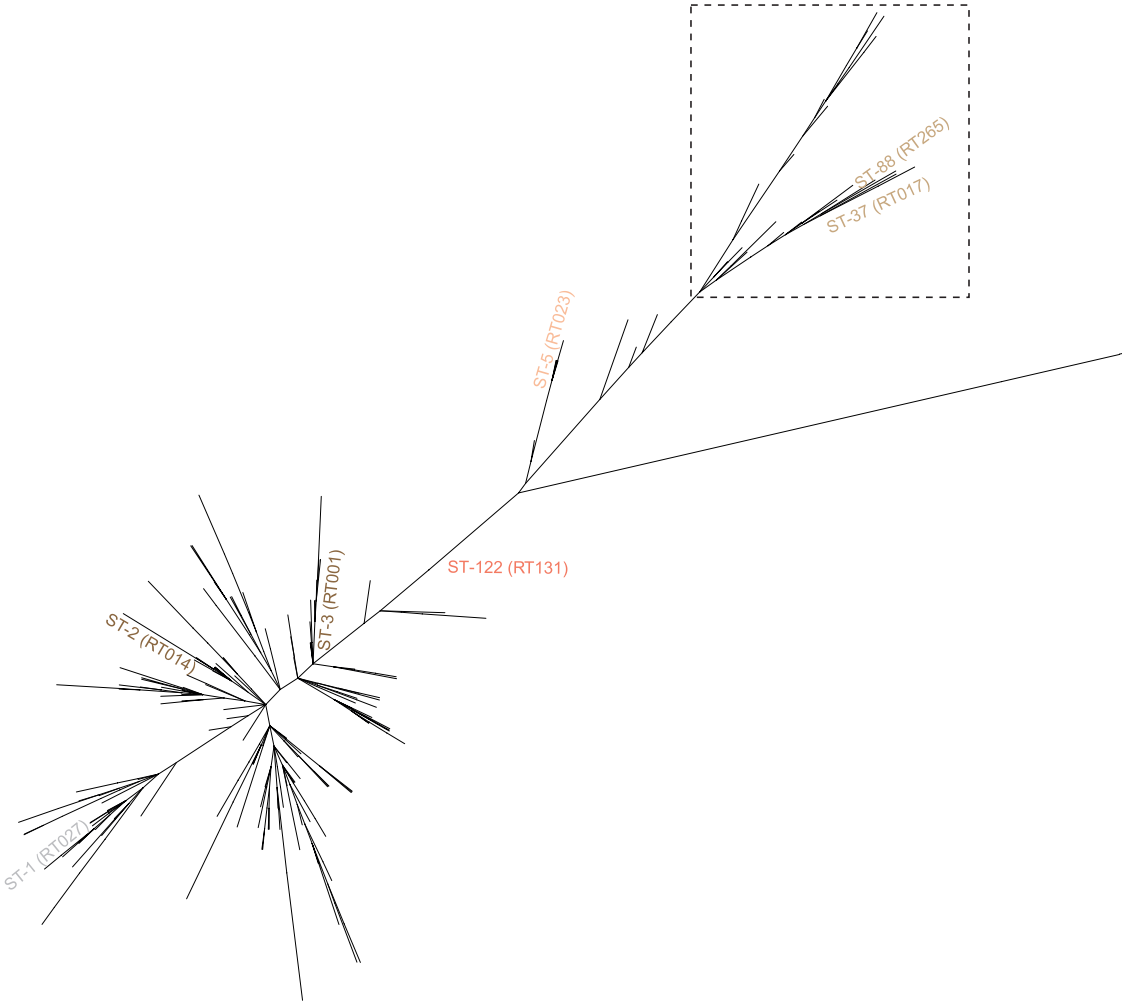


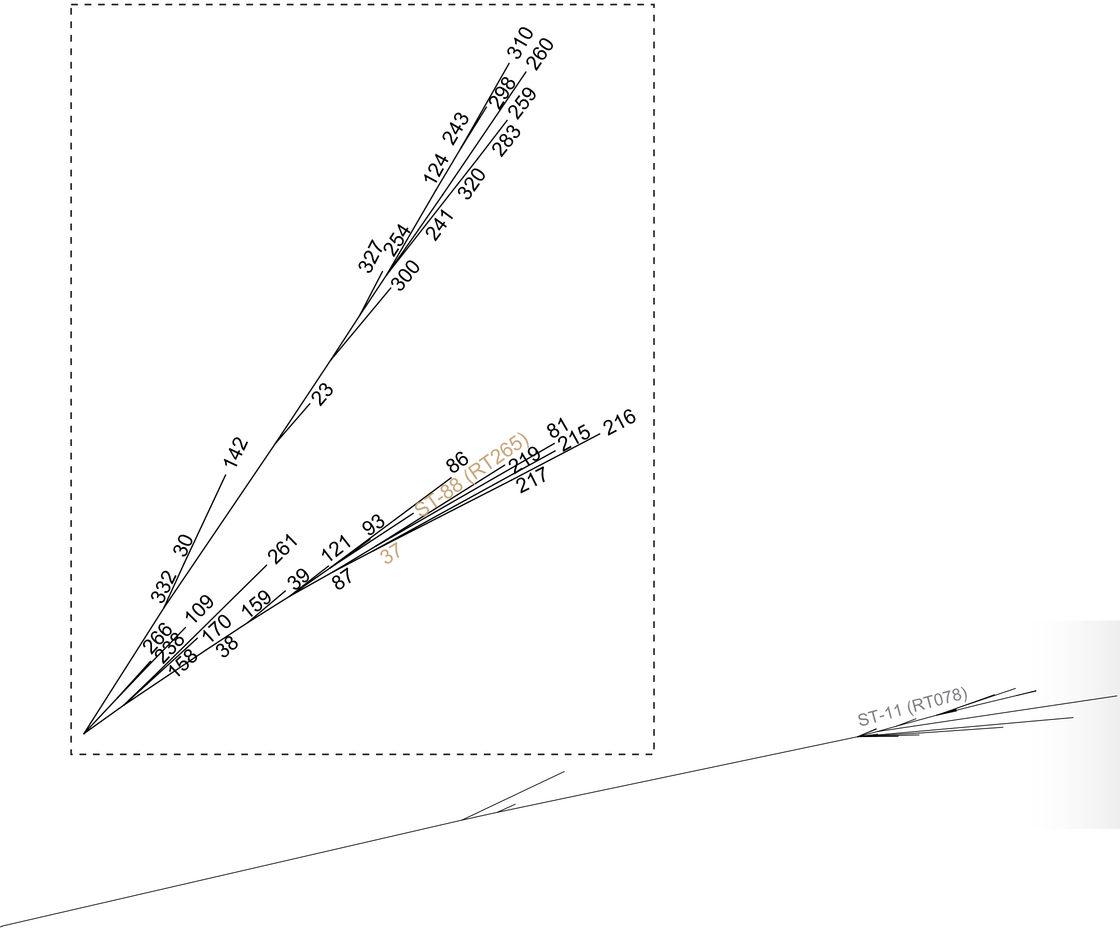
Supplementary Table 1. Numbers of reported paediatric CDIs per total number of CDIs, stratified by 12-month time period (May 2009-April 2015) and hospital, and primary CDI diagnostic test(s) applied.

Hospital type	Primary CDI diagnostic test(s)[a]	No. of reported children of all CDI cases (%)						
		2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	Total
University		20/677 (3)	16/750 (2)	26/748 (4)	27/750 (4)	22/825 (3)	24/941 (3)	135/4,691 (3)
U1	Toxin EIA, 3/15 NAAT	7/27 (26)	4/25 (16)	10/63 (16)	8/58 (14)	9/56 (16)	1/43 (2)	39/272 (14.3)
U2	Toxin EIA	2/30 (7)	4/38 (11)	2/18 (11)	2/32 (6)	2/37 (5)	1/27 (4)	13/182 (7.1)
U3	Toxin EIA, 1/11 NAAT, 2/15 GDH/toxin EIA	5/72 (7)	3/91 (3)	6/76 (8)	10/76 (13)	2/48 (4)	3/57 (5)	29/420 (6.9)
U4	Toxin EIA, 1/14 NAAT	0/25 (0)	1/32 (3)	1/45 (2)	1/24 (4)	1/51 (2)	4/78 (5)	8/255 (3.1)
U5	Toxin EIA, 11/13 NAAT	0/41 (0)	0/43 (0)	1/45 (2)	1/13 (8)	1/55 (2)	5/92 (5)	8/289 (2.8)
U6	GDH/toxin EIA					1/20 (5)	0/37 (0)	1/57 (1.8)
Primary and secondary care								
H1	CCA	3/29 (10)	0/10 (0)					3/39 (7.7)
H2	Toxin EIA, 4/14 GDH/toxin EIA	0/67 (0)	0/50 (0)	1/47 (2)	1/40 (3)	1/36 (3)	4/61 (7)	7/301 (2.3)
H3	Toxin EIA, 1/12 GDH/toxin EIA and NAAT	0/79 (0)	3/124 (2)	3/111 (3)	3/79 (4)	0/68 (0)	3/64 (5)	12/525 (2.3)
H4	Toxin EIA, 5/11 GDH/toxin EIA	1/28 (3.6)	1/7 (14)	0/19 (0)	0/32 (0)	0/13 (0)	0/28 (0)	2/127 (1.6)
H5	NAAT					1/36 (3)	0/48 (0)	1/84 (1.2)
H6	Toxin EIA, 1/14 GDH EIA	1/13 (8)	0/10 (0)	0/22 (0)	0/17 (0)	0/17 (0)	0/7 (0)	1/86 (1.2)
H7	GDH/toxin test, 8/10 NAAT	1/29 (3)	0/54 (0)	1/52 (2)	0/64 (0)	1/42 (2)	0/58 (0)	3/299 (1.0)
H8	Toxin EIA, 1/12 NAAT	0/53 (0)	0/55 (0)	0/13 (0)	0/29 (0)	2/26 (8)	0/21 (0)	2/197 (1.0)
H9	Toxin EIA, 3/12 NAAT	0/85 (0)	0/95 (0)	0/86 (0)	0/83 (0)	1/64 (2)	3/60 (5)	4/473 (0.8)
H10	Toxin EIA		0/19 (0)	1/66 (2)	0/87 (0)	0/61 (0)	0/52 (0)	1/285 (0.3)
H11	NAAT	0/48 (0)	0/46 (0)	0/43 (0)	1/52 (2)	0/70 (0)	0/37 (0)	1/296 (0.3)
H12	NAAT					0/33 (0)	0/52 (0)	0/85 (0.0)
H13	Toxin EIA, 11/14 GDH/toxin EIA	0/4 (0)	0/7 (0)	0/4 (0)	0/9 (0)	0/4 (0)	0/8 (0)	0/36 (0.0)
H14	Toxin EIA, 8/13 GDH/toxin EIA, 9/14 NAAT				0/18 (0)	0/54 (0)	0/38 (0)	0/110 (0.0)
H15	Unknown	0/7 (0)	0/5 (0)					0/12 (0.0)
H16	Toxin EIA	0/14 (0)	0/17 (0)	0/19 (0)	0/4 (0)			0/54 (0.0)
H17	Unknown	0/24 (0)	0/15 (0)					0/39 (0.0)
H18	Toxin EIA, 5/13 NAAT	0/2 (0)	0/7 (0)	0/8 (0)	0/12 (0)	0/6 (0)	0/10 (0)	0/45 (0.0)
H19	Toxin EIA			0/11 (0)	0/21 (0)	0/28 (0)	0/30 (0)	0/90 (0.0)
H20	NAAT						0/33 (0)	0/33 (0.0)

[a] If a change of the primary diagnostic test occurred, the month and year of implementation is reported.

Supplementary Figure 2 Phylogenetic tree of the genetic relatedness of PCR ribotype 265 (sequence type 88) to other *C. difficile* types. To include as many sequence types (STs) for the phylogenetic analysis as currently described, we downloaded (20-04-2016) the STs from the online *C. difficile* multilocus sequence typing (MLST) database (http://pubmlst.org/cdifficile/). In total, 319 STs were included in the phylogenetic analysis (Last updated: 2016-04-04). Nucleotide sequences of the seven housekeeping genes used for *C. difficile* MLST (Griffiths et al.) were concatenated; 219 SNPs were identified for the 319 STs. A maximum likelihood phylogeny was reconstructed using RaxML (ref: Stamatakis) with a general time reversible (GTR) model and gamma correction for among-site rate variation combined with 100 random bootstrap replicates (default). The STs numbers were removed to improve readability, only STs and corresponding PCR ribotypes (RTs) were kept for commonly found *C. difficile* types. The dashed window is enlarged to improve resolution for the lineage in which RT265 (ST-88) was located.




310
260
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ST-88 (RT265)
81
216
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219
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ST-11 (RT078)

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